Massachusetts Department of Public Health

State Laboratory Institute

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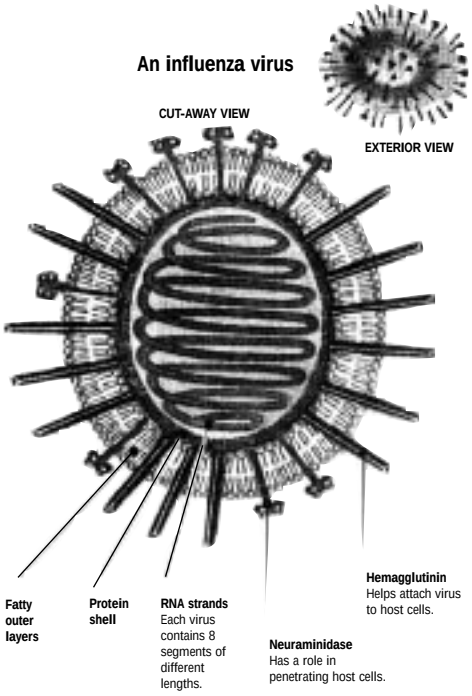
Influenza Surveillance for 1998-99

by Kristin Myers

The State Laboratory Institute (SLI) conducts surveillance for influenza each year and participates, through the U.S. Centers for Disease Control and Prevention (CDC), in the WHO Global Influenza Surveillance Program. The surveillance system is effective because of the commitment of physicians and hospital and private clinical laboratories throughout the state to provide early warning of clinical illness and submit specimens from patients to SLI. SLI provides timely data on the prevalence of influenza viruses and the antigenic changes in circulating strains of influenza in the community. This information aids public health and medical practitioners in their disease prevention activities. The 1998-99 season has begun in Massachusetts with the appearance of influenza B-Beijing-93-like virus on November 18 and influenza A (H3N2) Sydney-like virus on December 3, 1998.

Currently, three influenza strains of public health significance are found globally and are incorporated in the current influenza vaccine. The influenza viruses isolated this season in the United States have been characterized as similar to A/Sydney/05/97 (H3N2), A/Beijing/262/95 (H1N1), and B/Beijing/184/93, all components of the vaccine. Influenza types are based upon the viral nucleoproteins and matrix proteins, and subtypes are based primarily on hemagglutinin (HA) and neuraminidase (NA) membrane glycoproteins.

Proper specimen collection, handling, and transport are critical to successful influenza virus detection. Information for proper specimen collection is included with the kits provided by SLI for testing residents of Massachusetts. Specimens for isolation should be collected within 2 days of onset of symptoms and sent to SLI in viral transport media on cold packs as soon as possible. Specimens collected more than 2 days after onset of symptoms, or not maintained at proper temperatures, are unlikely to yield



Graphic: Reprinted Courtesy of The Boston Globe

virus. In the absence of an acceptable respiratory specimen, paired serum specimens can be tested for evidence of seroconversion to influenza and other respiratory viruses.

Influenza viruses infect cells of the respiratory tract. The virus exploits normal cellular systems to expand its population. The immune system senses the virus and responds. The response includes the clinical manifestations of fever, sore throat, myalgia, dry cough, headache, coryza or congestion, and malaise. Normally, the illness persists for up to two weeks, although in certain persons such as elderly or those afflicted with chronic

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PFGE

Molecular Laboratory among first to join national network for subtyping of *E. coli* O157:H7.

by Deborah Shea and Joseph Peppe

The State Laboratory Institute (SLI) was represented by Ralph Timperi, SLI Director, at a White House Ceremony in May at which Vice President Al Gore announced the launch of PulseNet, the CDC computer network that tracks food-borne illness. Massachusetts was one of 4 state public health laboratories initially designated as a regional testing site to perform pulsed field gel electrophoresis (PFGE). The regional laboratories perform PFGE according to a standard protocol and incorporate universal standards to assure comparable DNA patterns from PFGE analyses (*JAMA, 277:17*, 1998, 1337-40).

SLI receives isolates of *Escherichia coli* O157: H7 and other bacterial organisms causing food-borne diseases. Hospital and independent clinical laboratories are key partners in this disease surveillance system and regularly send isolates to SLI. These isolates are confirmed by biochemical tests, serotyped and then tested by PFGE. Laboratory-based surveillance using molecular

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illness, influenza can progress to life-threatening complications such as pneumonia.

From early October through mid-April, SLI accepts primary respiratory specimens for testing and typing, as well as positive isolates from area hospitals for confirmation and typing. Detection of influenza virus in cell cultures that support viral growth is definitive proof of a positive specimen; the resulting isolate is then typed and further subtyped using reagents provided by the CDC. Because this process can span several days, the laboratory employs rapid diagnostic methods for presumptive identification of positive specimens. Centrifugation-enhanced culture techniques using shell vials (coverslips coated with a cellular monolayer) permit the detection of influenza A or B in 24-48 hours, while other rapid tests allow for preliminary identification of influenza A in less than two hours. Because these tests sacrifice sensitivity, they are used as a supplement to the highly sensitive conventional tube cultures for influenza diagnosis.

SLI transmits data on antigenic characteristics of influenza isolates to CDC weekly. National surveillance data can be accessed through CDC's Web site at http://www.cdc.gov/ncidod/diseases/flu/weekly.htm. For information about influenza virus collection kits, please call the Influenza Coordinator at 617-983-6800. Questions regarding testing can be directed to the virus isolation laboratory at 617-983-6383.

Quality Assurance & Regulations

Analyte Specific Reagents and Home Brew Assays

by Dina Caloggero

The Food and Drug Administration (FDA) now regulates components of so called "home brew" assays. The Analyte Specific Reagent (ASR) rule took effect on November 23, 1998 and was published in the Federal Register, Vol. 62, No. 225, November 21, 1997, 62243-62260.

The ASR rule affects manufacturers of ASRs and clinical laboratories that utilize ASRs in their in-house or "home brew" assays. ASRs can be sold only to clinical laboratories regulated under the Clinical Laboratory Improvement Amendments of 1988 (CLIA), which are qualified to perform high complexity testing, and laboratories regulated under the Veteran's Health Administration Directive 1106.

The final rule defines an ASR as "antibodies, both polyclonal and monoclonal, specific receptor proteins, ligands, nucleic acid sequences, and similar reagents which, through specific binding or chemical reaction with substances in a specimen, are intended for use in a diagnostic application for identification and quantification of an individual chemical substance or ligand in biological specimens." Manufacturers of ASRs must comply with FDA requirements for Class I medical devices. That means ASRs are exempt from the FDA's 510(k) process but must meet registration and listing requirements, FDA good manufacturing practices (GMPs), and post market reporting rules.

Clinical laboratories using ASRs in their in-house assays must include a disclaimer on their test reports indicating that ASRs were used. The statement must read: "This test was developed and its performance characteristics determined by (laboratory name). It has not been cleared or approved by the US Food and Drug Administration."

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Grants, Projects & Publications

HIV Seroincidence Studies

by Barbara Werner

The HIV Laboratory and Serosurveillance Program was recently awarded funds as part of the Massachusetts HIV/AIDS Cooperative Agreement with the Centers for Disease Control and Prevention (CDC) to conduct HIV seroincidence studies. The HIV Laboratory will function as a Regional Laboratory performing testing for programs in other states as well. Seroincidence determinations will be based on a serologic testing algorithm described recently by Janssen and coauthors (JAMA. 1998;280:42-48). Persons reactive by a sensitive enzyme immunoasay (EIA) for anti-HIV, but non-reactive on a modified, less sensitive EIA, can be identified as recently infected. Incidence estimates can thus be based upon cross-sectional serosurveys, less biased and less expensive than longitudinal studies.

This testing strategy should be useful at the population level for estimating HIV-1 incidence, at the clinical level for identifying subjects with early infection for therapeutic intervention, and at the public health level for evaluating and targeting HIV prevention efforts.

The HIV Laboratory has provided testing in support of the Massachusetts Department of Public Health (MDPH) anonymous and confidential Counseling and Testing programs since the mid-1980s and for the CDC sponsored HIV seroprevalence studies since 1988.

PFGE

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subtyping of pathogens monitors trends in causes of illness, and rapidly identifies clusters of disease. When an outbreak is suspected, a 24-hour PFGE protocol is used to evaluate suspect specimens for genetic relatedness. When isolates are found with the "same" DNA pattern by PFGE analysis (interpreted as "indistinguishable" DNA patterns), epidemiologists can follow-up to confirm linkage between cases using additional information such as food histories. The specificity and shortened analytical time of the PFGE procedure permits early detection and action to control a disease outbreak and prevent further food-borne disease.

Local and multi-state outbreaks have been identified within a few days of the start of a widespread event using the PulseNet system. The national database allows testing centers to compare their DNA patterns in real time with patterns from other centers. If similar patterns are found in the database, epidemiologic data can be retrieved and contact made with epidemiologists from other affected states. Using PulseNet, multi-state outbreaks have been identified among contiguous states, e.g., suspected contaminated hamburger linked to cases in MA, NH, and CT and among distant states, e.g., suspected contaminated parsley linked to cases in MA, MN and WA.

At this time, *E. coli* O157:H7 and Salmonella typhimurium protocols have been validated and standardized among the regional laboratories. Salmonella typhimurium will be the next organism added to the PFGE national database. Although their protocols are not fully validated, other organisms have been analyzed by PFGE and have shown good comparability among the regional laboratories. The regional laboratories use the same equipment, reagents and protocols, which aid comparison of test results. SLI has analyzed isolates of Salmonella sp., Shigella sp., Listeria monocytogenes and Neisseria meningitidis and identified apparently related cases based on genetic similarity of the organisms and epidemiologic relationship (e.g., time, place and common source).

Pulsed field gel electrophoresis now is in widespread use as a tool in molecular epidemiology. Interpretation of DNA patterns following PFGE is becoming more refined as the central database provides more information on the natural prevalence and diversity of PFGE patterns.

PFGE is based on electrophoretic analysis of an enzyme digest of a bacterial suspension in an agarose gel utilizing a multi-directional electrical field. Bacteria are immobilized in agarose plugs, lysed and treated with a restriction endonuclease (RE) to cut the bacterial DNA into many fragments of assorted sizes. The RE cuts each DNA strand many times at recognition sites which typically are 5-6 base pairs long. Sometimes an isolate is analyzed using a second RE to confirm findings in the first analysis.

High resolution is obtained in PFGE due to the application of a consistently increasing switch time in the electrical field (pulsed field) being applied to the gel. Bacterial fragments move through the gel at a speed inversely proportional to their size. Following electrophoresis, gels are stained to visualize the "bands" of similar sized fragments, and the patterns are photographed. The photograph is scanned into a computer and digitized to permit software-assisted analysis and comparison of patterns.

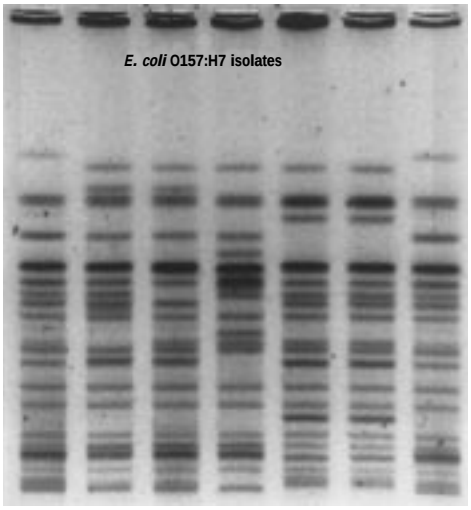

Figure 1. PFGE Patterns for *E. coli* O157:H7 Isolates

Each PFGE run on a single gel sheet can contain up to 15 "lanes", usually with 12 individual samples and 3 standards. An example of a stained gel is shown in Figure 1, which is a run of *E. coli* O157:H7 isolates. Lanes 1 and 7 are global standard isolates of *E. coli* O157: H7. Lanes 2, 3, 4, 5, and 6 are isolates of *E. coli* O157:H7 from Massachusetts and Vermont. The specimens in lanes 5 and 6 were identified as indistinguishable isolates based on PFGE patterns.

Although interpretation criteria vary depending on the organism and the epidemiologic situation, common criteria have been established as a guideline for interpreting test results.

One set of criteria for interpretation has been used widely in PFGE analysis (Tenover et al, J. Clin. Micro., 33, 2233, 1995). These criteria are shown in Table 1.

Table 1. Criteria for Interpreting PFGE Patterns

Category	No. of genetic differences	Typical no. of fragment differences	Epidemiologic relatedness of the isolate in relation to the outbreak
Indistinguishable	0	0	Part of outbreak
Closely related	1	2-3	Probable
Possibly related	2	4-6	Possible
Different	≥ 3	≥ 7	Not part of outbreak

Outbreak strains are isolates of the same species that are epidemiologically and genetically related. Such isolates are presumed to be clonally related when they exhibit common phenotypes and genotypes and are temporally related.

Endemic strains are isolates that are found frequently from infected patients in a health care setting or community and are indistinguishable or closely related by typing methods, but have no demonstrable epidemiologic link. Such isolates are presumed to be clonally related, but their common origin may be more temporally distant compared to outbreak strains.

Knowledge of PFGE gel patterns is growing rapidly as data and information from thousands of test results run under standardized protocols are compiled through the regional public health laboratory system. This powerful laboratory method is aiding disease control activities and will assist the assurance of a safe food supply.

For additional information about the PFGE testing program at SLI, contact deborah.shea@state.ma.us.

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for the electronic version of this newsletter and other information about the State Laboratory Institute: www.state.ma.us/dph/sli.htm.

Laboratory Training Activities

State Laboratory Institute 1999 Public Health Teleconference Series on Infectious Diseases:

Local and national experts provide up to date information on current issues and answer questions from participants via conference telephone. The cost for each conference is $25 per site. Call (617) 983-6284 to register and obtain information on CEUs.

May 25	Hepatitis C: The Facts and The Issues
June 22	Preventing the Spread of Vancomycin Resistance
October 19	Influenza: Preparing for the Pandemic?
November 16	*Chlamydia trachomatis* - With An Eye on Amplification

For a complete listing of upcoming training workshops and seminars, contact the State Laboratory Training Coordinator, *Garry R. Greer, BS, (617) 983-6608, E-mail: garry.greer@state.ma.us*.

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